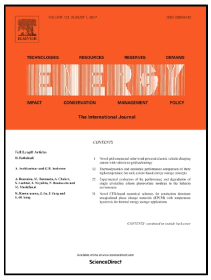
Title	Comparison of pre-treatments to reduce salinity and enhance biomethane yields of Laminaria digitata harvested in different seasons
Authors	Tabassum, Muhammad Rizwan;Xia, Ao;Murphy, Jerry D.
Publication date	2017-08-16
Original Citation	Tabassum, M. R., Xia, A. and Murphy, J. D. (2017) 'Comparison of pre-treatments to reduce salinity and enhance biomethane yields of Laminaria digitata harvested in different seasons', Energy, 140(1), pp. 546-551. doi:10.1016/j.energy.2017.08.070
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.energy.2017.08.070
Rights	© 2017, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2023-05-05 20:50:47
Item downloaded from	http://hdl.handle.net/10468/4777

Accepted Manuscript



Comparison of pre-treatments to reduce salinity and enhance biomethane yields of *Laminaria digitata* harvested in different seasons

Muhammad Rizwan Tabassum, Ao Xia, Jerry D. Murphy

PII:	S0360-5442(17)31446-9
DOI:	10.1016/j.energy.2017.08.070
Reference:	EGY 11439
To appear in:	*Energy*
Received Date:	25 July 2016
Revised Date:	29 June 2017
Accepted Date:	15 August 2017

Please cite this article as: Muhammad Rizwan Tabassum, Ao Xia, Jerry D. Murphy, Comparison of pre-treatments to reduce salinity and enhance biomethane yields of *Laminaria digitata* harvested in different seasons, *Energy* (2017), doi: 10.1016/j.energy.2017.08.070

Comparison of pre-treatments to reduce salinity and enhance biomethane yields of *Laminaria digitata* harvested in different seasons

Muhammad Rizwan Tabassum [a], Ao Xia [b*], and Jerry D. Murphy [a, c]

a. MaREI Centre, Environmental Research Institute, University College Cork, Cork, Ireland
b. Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Chongqing 400044, China
c. School of Engineering, University College Cork, Cork, Ireland

Abstract

Pre-treatment can enhance anaerobic digestion of seaweed; however, seasonal variation in the biochemical composition of seaweed has a significant impact on the pre-treatment effect. In this study, various pre-treatments were employed for the brown seaweed *Laminaria digitata* harvested in March (with high ash content and low carbon to nitrogen (C:N) ratio) and September (with low ash content and high C:N ratio). Washing of *L. digitata* harvested in March with hot water (defined as 40 °C) removed 54% of the ash and improved the volatile solids (VS) content by 31% leading to an improved biomethane yield of 282 L CH_4 kg VS^{-1}. This pre-treatment affected a 16% increase in biodegradability, reduced salt accumulation in the digestate by 54%, and increased specific methane yield per wet weight by 25%. This level of effect was not noted for seaweed harvested in September, when the biodegradability is higher.

Keywords: *Laminaria digitata*; Seaweed; Pre-treatment; Anaerobic digestion; Biomethane

* Corresponding author at: Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Chongqing 400044, China. E-mail address: aoxia@cqu.edu.cn (Ao Xia).

1. Introduction

Anaerobic digestion is a well-established technology with a potentially higher gross energy yield per hectare as compared to liquid biofuel land-based biomass systems; seaweed biomethane does not compete with food for arable land [1-6]. Brown seaweeds are reported as an abundant marine bioresource in Irish waters [7]. The feedstock received more attention after the European Parliament communication that advanced biofuels (such as from seaweed) should represent at least 1.25% of renewable energy supply in transport (RES-T) [8].

Brown seaweed harvested during different times round the year displays a significant seasonal variation in biochemistry that has a significant influence on the digestibility of the seaweed for biogas production [4, 9, 10]. According to seasonal variation studies, autumn was considered the best harvesting period for biogas production [11]. Biodegradability of *Laminaria digitata* is lower in winter and spring; this can be attributed to lower levels of readily digestible carbohydrates (such as laminarin and mannitol), higher ash contents (mostly salts) and a higher level of process inhibitors [9, 12]. Ash is the significant component of brown seaweed that changes greatly through the whole year and can be up to 35% of dry weight [9, 13]. It has been suggested that due to high ash (salt) content in Spring, the seaweed is not recommended for biogas production [4]. Accumulation of salts in long-term digestion can be problematic [14]; this problem is heightened when the seaweed is harvested in Winter or early Spring [4, 15]. However, if significant salt removal of the feedstock could be achieved (with effective low energy input pre-treatments), the possibility of utilization of the Spring harvested biogas to produce biomethane could be closer to levels achieved from the autumn harvested seaweed. To increase the digestibility and degradability of brown seaweed (particularly in Spring), some pre-treatment may aid biogas production; however, due to the absence of cellulose and lignin, harsh pre-treatment may not be required [16].

The literature outlines pre-treatments employed on biomass such as physical (washing) [17], mechanical (size reduction by cutting, chopping, beating and maceration) [18], chemical [19], hydrothermal (heating) [20] and thermochemical processes [17]. Mechanical pre-treatment is considered as the most suitable approach for seaweed biogas production [5, 21], whereas chemical pre-treatment was found to be inhibitory [17]. Beating pre-treatment was identified as an efficient method to enhance the specific methane yield [11]. The brown seaweed *L. digitata* was found more suitable for beating pre-treatment to achieve a high specific methane yield than *Ascophyllum nodosum* [22]. Nevertheless, mechanical pre-treatments such as ball milling and beating have been described as high energy input pre-treatment methods for seaweed [23].These methods involve multi-step processing (cutting, drying, milling and sieving prior to processing) and include for installation of energy intensive machinery [24]. Moreover, these mechanical pre-treatment methods do not lead to a reduction in salt accumulation in the bioreactor during digestion. It was revealed that deionized water and acid pre-treatment could wash away the salt (ash), including sodium, potassium, magnesium, calcium and aluminium metal ions from the seaweed *Enteromorpha* [25]. Therefore, energy-saving pre-treatment methods (such as hot washing of seaweed and maceration) may be applied to brown seaweed to remove salts and other inhibitory components to improve subsequent biogas production.

The authors suggest two significant gaps in the state of the art of seaweed biomethane: assessment of effective low energy input pre-treatments to reduce the effect of salt accumulation (and associated inhibition) in digestion; and the variation in the effect of these pre-treatments on seaweeds harvested in different seasons. The innovation of the current work is to highlight the effect of combined but simple low energy pre-treatment methodologies (such as washing and maceration) on biomethane yields from *L. digitata*, (the dominant brown seaweed in the Atlantic waters surrounding the UK and Ireland) harvested in spring (when it is slow to biodegrade) and in autumn (when biodegradability is highest). The objectives of this study are to:

- Examine the effect of pre-treatments on spring and autumn harvests of *L. digitata*;
- Study the improvement in the biomethane yield and process dynamics;
- Investigate the effect of pre-treatment on salt accumulation in the batch reactor.

2. Materials and Methods

2.1 Collection and processing of *L. digitata* for pre-treatments

L. digitata was collected from Roaring Water Bay, Co. Cork, in the south of Ireland (51°N, -9°E) during March (spring in the northern hemisphere) and September (autumn in the northern hemisphere). Combinations of various pre-treatments were applied to investigate the effects on the biomethane yields based on seasonal variation in chemical composition. Washing pre-treatment of the seaweed was carried out at two different temperatures, namely: 15 ± 1 °C described hereafter as cold water; and 40 ± 1 °C described as hot water. The fresh fronds (blades) of the seaweed were washed with cold water for 3 minutes to remove any foreign particles. After washing, two mechanical pre-treatments (cutting and maceration) were applied to reduce the particle size. The washed samples were cut by scissors to a size of approximately 4 cm (hereafter termed CC: cold cut). Some samples were subsequently were macerated in a Buffalo macerator to further reduce the size to less than 4 mm (hereafter termed CM: cold macerated).

The seaweed samples (fronds only) were washed with hot water for 3 minutes, and then cut with scissors (termed HC: Hot cut) to allow comparison with CC. The samples washed with hot water were macerated (termed HM: hot macerated) to the same size (4 mm) to compare it with CM. Fresh unwashed samples were directly cut into a particle size of approximately 4 cm and used as the control group (referred to as untreated). All samples were frozen at -20 °C before analysis and before assessment for biomethane potential (BMP).

2.2 Analytical methods

Total solid (TS), volatile solid (VS) and ash were analysed by using the standard method of drying of the seaweed for 24 hours at 105 °C and subsequent combustion for 2 hours at 550 °C [26]. Elemental analysis was assessed by preparing the seaweed samples through drying at 105 °C for 24 hours and then grinding to pass through a 500 µm sieve. Dried samples were analysed for carbon, hydrogen, nitrogen and oxygen (oxygen calculated by difference) using a CE 440 elemental analyser.

2.3 Anaerobic digestion of the seaweed

The theoretical methane potential (TMP) was calculated by inserting the relative ratios of carbon, hydrogen and oxygen in the seaweed composition into the Buswell equation (Eq. (1)). The output from this equation provides a maximum potential methane yield [27]. The molar volume of the gases was taken as 22.14 L at 0 °C and 1 atm.

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \quad \text{Eq. (1)}$$

The inoculum was sourced from lab-scale continuous stirred-tank reactors (operated at 37 °C), processing various substrates such as grass, dairy slurry and seaweed. The BMP tests of the seaweed were conducted in a bioprocess system (Bioprocess AMPTS II® system). The Bioprocess AMPTS system is an automated methane potential test system with output to a software package. The BMP system has the capacity to accommodate 15 glass bottles, which served as batch digesters. Each glass bottle has a total volume of 650 ml with a working volume of 400 ml. All glass bottles were sealed with rubber corks and were purged with nitrogen gas for five minutes to create an anoxic environment. The bottles contained a continuous mixing system operating at 30 rpm and were kept at 37 °C using a water bath. Carbon dioxide and hydrogen sulphide were removed by passing the gas through 3 M sodium hydroxide solution. The gas flow was measured by a gas tipping device and the volume was automatically normalized to standard temperature (0 °C) and pressure (1 atm) and zero moisture content by the Bioprocess AMPST II® system. The substrate to inoculum ratio (S:I) on a VS basis, of 1:2 was used [28, 29]. To calculate the specific biomethane production, the total average biomethane produced by the inoculum was subtracted from the average biomethane produced by each sample [30]. Batch trials were conducted in triplicate, and the results were expressed as mean value ± standard deviation. Salinity (g/L) and pH of the batch digestion processes were also recorded before and after each BMP assay to investigate the effect of pre-treatment on the reaction performance and the gas yield.

2.4 Process dynamics and statistical analysis

The study of the process dynamics is beneficial to facilitate an understanding of the changes in the biodegradability and in the rate of biodegradability of the substrate before and after pre-treatment. The kinetic parameters such as a change in the decay constant (days^{-1}), maximum yield (Y_{max}) and half-life (days) were obtained by taking data from the cumulative methane production curves (after 30 days) and analysing in MATLAB software through a first order differential equation as described previously [4, 31]. The biodegradability index (BI) was defined as the ratio of the BMP yield to the theoretical value as expressed by the TMP (from Eq. (1)).

Statistical significance of each pre-treatment was determined through the use of statistical software (SPSS, IBM NY, USA). Analysis of variance (ANOVA) was performed to examine the effect of various pre-treatments on different parameters (such as ash removal, improvement in gas yields and enhancement in bio-degradability of the substrate). The significance level was determined by multiple comparisons (Post Hoc test).

3. Results and Discussion

3.1 Effect of pre-treatment on the seaweed composition

L. digitata was characterized for compositional and elemental analyses (Table 1). It should be noted that the untreated March seaweed has a ratio of ash to volatile solids (A:V) of 0.51 compared to 0.24 for September and a C:N of 8.2 as compared to the September sample of 39.4. Ideally the C:N should be greater than 20 for optimal digestion performance [4, 6]. It may be stated that the untreated March seaweed is not as suitable for anaerobic digestion as the September sample. March and September harvests of the seaweed were compared before and after each pre-treatment (Table 1). Pre-treatment can remove the attached salts of *L. digitata*, thereby decreasing the ash content leading to a change in the VS content when expressed as a percentage of fresh weight. After pre-treatment, it was revealed that washing with cold water did not remove a substantial amount of salts and hence did not improve the VS composition of the substrate. However, the VS content of the seaweed harvested in March was improved from 6.5% to 7.0% when washed with hot water and macerated (HM) to a particle size of less than 4 mm (Table 1). For samples harvested in March, HM pre-treatment succeeded in reducing ash content from 33.3% to 15.6%, resulting in increasing organic matter content from 66.7% to 84.4% and decreasing A:V ratio from 0.51 to 0.19. The substantial removal of ash (salt) content should make the seaweed more degradable [4, 12]. Significance of salt (ash) removal up to 54% in this study (Fig. 1) can be compared with previous studies in which pre-treatment of seaweed biomass with deionized water and with acid was found helpful in salt removal [25]. Removal of ash and improvement of VS content for the March harvest can be advantageous for long-term continuous digestion, as salt accumulation was reported as high in batch and continuous digestion processes [4, 15, 16].

However, for samples harvested in September, the VS content is relatively stable. This can be attributed to the fact that the removal of salt may be accompanied by the removal of soluble organic materials, such as mannitol, which is abundant in *L. digitata* harvested in autumn [12].

The C:N ratio and the A:V ratio are considered as the key factors for digestion of seaweed [4]. Washing with cold water of the March sample did not lead to a rise in the C:N ratio; however, washing with hot water did. Hot water pre-treatment may cause the removal of nitrogenous compounds such as proteins, lectins and alkaloids [32] and ultimately lead to an improvement in the C:N ratio (from 8.2 to 13.8 in this study). Hot water pre-treatment also facilitated the reduction in the A:V ratio from 0.51 to 0.19 in the March sample due to substantial removal of ash (Table 1). The percentage improvement of the C:N ratio was greater in the March harvest than the September harvest due to the higher content of nitrogenous compounds in the March seaweed than the September seaweed [4]. Removal of ash, improvement of VS content and increase of the C:N ratio for the March harvest is advantageous for long-term digestion, as salt accumulation was reported as significant in batch and continuous digestion processes and potentially inhibitory to digestion at elevated levels [4, 15]. The C:N ratio of the seaweeds in this current study were either lower (March) or higher (September) than the reported optimum values (20 to 30), however, no acid accumulation was observed during the digestion as evidenced from a buffered pH (ranged from 7.0 to 7.7) after the 30-day trial.

3.2 Impact of pre-treatment on the biogas yield

Seaweed harvested in spring (March) displayed a higher ash content and lower organic matter content, which would significantly reduce biomethane yield [4]. To investigate the effect of pre-treatment on the gas yield, various pre-treatments were designed and compared. The BMP results revealed that size reduction (4 cm and 4 mm) after washing with cold water had little effect on the specific methane yield expressed as L CH_4 kg VS^{-1}, when compared to the same size reduction after hot washing (Table 2).

It was observed (Table 2) that the effect of hot washing was more significant on the March harvest (from 245 L CH_4 kg VS^{-1} to 283 L CH_4 kg VS^{-1}) than the September harvest (from 280 L CH_4 kg VS^{-1} to 326 L CH_4 kg VS^{-1}). The rationale for this difference can be explained by the difference in seasonal chemical composition. Seaweed harvested in March had high ash content as compared to September (Table 1), hence, there is more significant potential for ash removal [4].

Particle size reduction of dried seaweed was reported as an effective pre-treatment for biogas production from brown seaweeds [33]; however, drying is considered to be an energy intensive process on an industrial scale. In the current trials maceration to a particle size of 4 mm is deemed unnecessary as compared to size reduction by scissors to 4 cm particle size (Table 2). The gas yield was almost the same for both particle sizes for the March harvest (282 L CH_4 kg VS^{-1} and 283 L CH_4 kg VS^{-1}). However, the specific methane yield calculated based on wet weight highlights that maceration is an optimal step for the March harvest (Table 2). The specific yield, improved by 25% (to 20 m^3 CH_4t wwt^{-1}) for maceration after hot washing, as compared to the sample cut by scissors (15 m^3 CH_4 t wwt^{-1}) (Table 2). On the other hand, size reduction by scissors (4 cm) after hot washing decreased the specific methane yield compared to the untreated sample (16 m^3 CH_4 $twwt^{-1}$). This may be attributed to material loss during manual cutting with scissors and can be avoided on an industrial level using mechanical instruments.

The current study can be correlated with other mechanical pre-treatments such as ball milling and beating [23]. However, these techniques involved multi-step processing (prior to the digestion) and require more intensive energy input [24]. Additionally, such mechanical pre-treatment methods have no impact on the issue of accumulation of salts in the bioreactor during digestion. The current pre-treatment method is unique in that it is simple (hot washing and subsequent maceration) but also successfully reduces the inhibitory effect of salt accumulation in bioreactors.

The exact mechanism or possible reason behind the hot washing pre-treatment is not fully known. However, it may be explained that removal of salts associated with the cell wall polysaccharide alginate (such as sodium, potassium, magnesium, etc.) along with some inhibitory components changed the biochemistry of the seaweed and made the substrate more degradable. This has also been reported in a previous study that described the effect of washing the seaweed biomass with deionized water [25]. Scanning electron microscopy

(SEM) confirmed that the seaweed biomass surfaces were eroded by removing salt (ash) contents and extracts [25]. Other potential inhibitors, such as poly-phenols, sulphated polysaccharides (fucoidan), fucoxanthin and associated epiphytes, can be removed via hot washing [32]. It was reported that washing can also remove the epiphytes, which have high antimicrobial activities, from the surface of the seaweed [32].

3.3 Effect of pre-treatment on the key process parameters

Ash content, organic matter content and C:N ratio are the key process parameters, which can affect biodegradability and the gas yield. Seaweed with lower ash content, higher organic matter content, and C:N ratio in the optimum range are advantageous for biogas production [4, 9].

Ash content in marine biomass can be an issue in long-term anaerobic digestion through accumulation of salts in the digester. Tabassum et al., [4] charted the seasonal variation of *L. digitata* through the twelve months of the year and found the salt build-up was higher in winter and spring samples of seaweed as compared to summer and autumn samples. In this work, hot water washing reduced the ash content by 54% (March) and 31% (September) when cut to a particle size of 4 cm by scissors; values of 47% and 27% were achieved respectively when macerated (Fig. 1). Ash removal resulted in an increased organic matter content of 31% and 8% in March and September, respectively when the seaweed was cut by scissors after hot water washing.

BI (defined as the ratio of the BMP yield to the theoretical yield) explains the process efficiency in terms of degradability of the substrate in the reactor. BI improved from 0.52 to 0.61 of the seaweed harvest in March while it was increased from 0.62 to 0.76 for the September harvest (Table 2). The substrate was 16% and 23% more biodegradable as compared to untreated seaweed for March and September harvest, respectively (Fig. 1). The higher BI in September as compared to the March harvest may be due to higher concentrations of easily degradable organic matter content (such as mannitol) in the substrate [9, 12].

Accumulation of salts was recorded before and after pre-treatments to examine the reduction of salts in the reactor. Salinity and the A:V ratio were reported as key factors affecting the gas yield during different harvesting seasons. Higher values of salinity and A:V led to lower values of biomethane production [4]. The salinity of seaweed was subtracted from the salinity of inoculum (6.85 ± 0.46 g/L) to calculate the salinity increase in the batch digestion due to the seaweed only. It was observed that hot water pre-treatment successfully resulted in lowering the A:V ratio and salinity (Fig. 2) as compared to cold water pre-treatment. However, the reduction in the percentage salinity was comparatively higher for the March harvest (Fig. 2). A low biomethane yield was expected at a high salinity level [34-36]. Application of hot water washing (hot cut) as a pre-treatment technology before anaerobic digestion of the seaweed resulted in 54% less salt accumulation in the reactor as compared to the untreated March harvest.

After the process parameter studies, it was revealed that pre-treatment has the greatest impact on the March harvest. While, for the September harvest, the impact of pre-treatment is lower due to the already higher organic matter content, lower ash content and lower levels of inhibitory compounds in the substrate as compared to the March harvest.

3.4 Process dynamics and statistical analysis

The changes in the process dynamics after each pre-treatment are listed in Table 3. Maceration after hot water washing (HM) indicated significant kinetic decay increase as the

k value doubled from 0.10 to 0.20 for the March harvest and the biomass was degraded efficiently (half-life was shortened from 6.8 to 3.4 days). This may be attributed to the removal of a substantial amount of inhibitory compounds (such as polyphenols) from the substrate that may be responsible for slower degradation of the seaweed in the untreated sample [37]. The decay values as the result of other pre-treatments for the same harvest remained close to the untreated sample (Table 3). The decay constants of *L. digitata* were comparable with those of perennial ryegrass, food waste, and brown seaweed reported previously in the range 0.11 to 0.19 [30, 38, 39]. After employing the HM pre-treatment for the March sample, the decay constant was improved by 100%, whereas the half-life methane production was reduced by 49%.

The half-life of biomethane production (T_{50}) from untreated seaweed was reported as 4-5 days in summer and 6-9 days in winter [3] probably due to the higher concentration of easily degradable laminarin and mannitol in autumn[9]. Improvement in kinetic parameters for the March harvest (after pre-treatments) delimited the utilization of the substrate for biogas production and may reduce the retention time for digestion of the substrate to less than 20 days, which is suggested sufficient for long-term continuous digestion [16, 30].

The experimental results were supported by statistical significance by conducting an ANOVA analysis. Multiple comparisons results from one-way ANOVA indicated that pre-treatment applied to the March harvest were significant ($F=8.39$, $P < 0.001$) as compared to the September harvest ($F=1.68$, $P < 0.23$). The change or improvement in the specific methane yield (of March harvest) was also found statistically significant ($F=11.56$, $P < 0.001$) while for the seaweed harvested in the September harvest, the values were not statistically significant ($F=1.68$, $P < 0.23$). The samples harvested in March were analysed further in comparison with different factors affecting the methane yield. These factors were particle size (4 mm and 4 cm) and washing method (hot water and cold water) in comparison with the removal of salts (ash), biodegradability and ultimately the BMP enhancement. After comparison, it was revealed that the most significant factor for the process was the A:V ratio ($F = 11.97$ and $P < 0.001$) and the ash content ($F = 14.09$ and $P < 0.001$). Particle size and washing method comparison results indicated that the most significant pre-treatment was maceration to a particle size of 4 mm after hot washing ($P < 0.002$).

After comparison of statistical results, it can be concluded that maceration after hot washing was the most efficient pre-treatment method to enhance the methane yield by substantial removal of salts (ash) from the substrate. However, optimization of pre-treatment time and temperature for seaweed is necessary to further improve the performance of biogas production.

4. Conclusions

Maceration of the brown seaweed after washing with cold water has little impact on the gas yield as compared to washing with hot water. Hot washing pre-treatment resulted in higher ash content removal from the March harvest than the September harvest. Scissor cutting after hot washing yielded 16% higher biomethane by removing 54% ash with an improvement of 31% of VS content. Maceration after hot washing pre-treatment significantly achieved a 25% higher specific methane yield per unit wet weight compared to the untreated sample. However, hot washing requires optimization of pre-treatment time and temperature to facilitate the continuous supply of the seaweed even in March for biogas production.

Acknowledgements

This material is based upon works supported by the Science Foundation Ireland (SFI) under Grant No. 12/RC/2302. Researchers are employed by the SFI centre, MaREI. Gas Networks Ireland (GNI) co-founded the work through funding of the Gas Innovation Group. Ervia also co-funded the work. Dr. Ao Xia acknowledges Chongqing University for the start-up funds under the "One Hundred Talents Program".

References

[1] Gebrezgabher SA, Meuwissen MPM, Prins BAM, Lansink AGJMO. Economic analysis of anaerobic digestion—A case of Green power biogas plant in The Netherlands. NJAS-Wagen J Life Sci 2010;57:109-15.

[2] Tenenbaum DJ. Food vs. Fuel: Diversion of crops could cause more hunger. Environ Health Perspect 2008;116:A254-A7.

[3] Gurung A, Van Ginkel SW, Kang W-C, Qambrani NA, Oh S-E. Evaluation of marine biomass as a source of methane in batch tests: A lab-scale study. Energy 2012;43:396-401.

[4] Tabassum MR, Xia A, Murphy JD. The effect of seasonal variation on biomethane production from seaweed and on application as a gaseous transport biofuel. Bioresour Technol 2016;209:213-9.

[5] Tedesco S, Marrero Barroso T, Olabi AG. Optimization of mechanical pre-treatment of *Laminariaceae* spp. biomass-derived biogas. Renew Energy 2014;62:527-34.

[6] Alaswad A, Dassisti M, Prescott T, Olabi AG. Technologies and developments of third generation biofuel production. Renew Sust Energ Rev 2015;51:1446-60.

[7] Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ. Threats and knowledge gaps for ecosystem services provided by kelp forests: A northeast Atlantic perspective. Ecol Evol 2013;3:4016-38.

[8] Parliment E. Environment Committee backs switchover to advanced biofuels Press release - Environment. available at http://wwweuroparleuropaeu/news/en/news-room/content/20150223IPR24714/html/Environment-Committee-backs-switchover-to-advanced-biofuels 2015.

[9] Adams JM, Ross AB, Anastasakis K, Hodgson EM, Gallagher JA, Jones JM, et al. Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion. Bioresour Technol 2011;102:226-34.

[10] Tabassum MR, Xia A, Murphy JD. Seasonal variation of chemical composition and biomethane production from the brown seaweed *Ascophyllum nodosum*. Bioresour Technol 2016;216:219-26.

[11] Montingelli ME, Benyounis KY, Quilty B, Stokes J, Olabi AG. Optimisation of biogas production from the macroalgae Laminaria sp at different periods of harvesting in Ireland. Appl Energy 2016;177:671-82.

[12] Adams JM, Toop TA, Donnison IS, Gallagher JA. Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresour Technol 2011;102:9976-84.

[13] Schiener P, Black K, Stanley M, Green D. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 2015;27:363-73.

[14] Gao K, McKinley K. Use of macroalgae for marine biomass production and CO_2 remediation: a review. J Appl Phycol 1994;6:45-60.

[15] Allen E, Wall DM, Herrmann C, Murphy JD. Investigation of the optimal percentage of green seaweed that may be co-digested with dairy slurry to produce gaseous biofuel. Bioresour Technol 2014;170:436-44.

[16] Tabassum MR, Xia A, Murphy JD. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. Renew Sust Energ Rev 2017;68:136-46.

[17] Oliveira JV, Alves MM, Costa JC. Design of experiments to assess pre-treatment and co-digestion strategies that optimize biogas production from macroalgae *Gracilaria vermiculophylla*. Bioresour Technol 2014;162:323-30.

[18] Nielsen HBH, S. Anaerobic digestion of macroalgae: Methane potentials, pre-treatment, inhibition and co-digestion. Water Sci Technol 2011;64:1723-9.

[19] Borines MG, de Leon RL, Cuello JL. Bioethanol production from the macroalgae *Sargassum* spp. Bioresour Technol 2013;138:22-9.

[20] Schultz-Jensen N, Thygesen A, Leipold F, Thomsen ST, Roslander C, Lilholt H, et al. Pretreatment of the macroalgae *Chaetomorpha linum* for the production of bioethanol--comparison of five pretreatment technologies. Bioresour Technol 2013;140:36-42.

[21] Tedesco S, Benyounis KY, Olabi AG. Mechanical pretreatment effects on macroalgae-derived biogas production in co-digestion with sludge in Ireland. Energy 2013;61:27-33.

[22] Montingelli ME, Benyounis KY, Quilty B, Stokes J, Olabi AG. Influence of mechanical pretreatment and organic concentration of Irish brown seaweed for methane production. Energy 2017;118:1079-89.

[23] Rodriguez C, Alaswad A, Mooney J, Prescott T, Olabi AG. Pre-treatment techniques used for anaerobic digestion of algae. Fuel Process Technol 2015;138:765-79.

[24] Montingelli ME, Benyounis KY, Stokes J, Olabi AG. Pretreatment of macroalgal biomass for biogas production. Energy Convers Manage 2016;108:202-9.

[25] Hu Y, Wang S, Wang Q, He Z, Lin X, Xu S, et al. Effect of different pretreatments on the thermal degradation of seaweed biomass. Proc Combust Inst 2017;36:2271-81.

[26] Xia A, Jacob A, Tabassum MR, Herrmann C, Murphy JD. Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro- and micro-algae. Bioresour Technol 2016;205:118-25.

[27] Buswell AM BC. The relation between the chemical composition of organic matter and the quality and quantity of gas produced during sludge digestion. Sew Works J 1932;4(3):454-60.

[28] Chynoweth DP, Turick CE, Owens JM, Jerger DE, Peck MW. Biochemical methane potential of biomass and waste feedstocks. Biomass Bioenerg 1993;5:95-111.

[29] Angelidaki I, Alves M, Bolzonella D, Borzacconi L, Campos J, Guwy A, et al. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Sci Technol 2009;59:927-34.

[30] Allen E, Wall DM, Herrmann C, Xia A, Murphy JD. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? Energy 2015;81:352-60.

[31] Nopharatana A, Pullammanappallil PC, Clarke WP. Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. Waste Manage 2007;27:595-603.

[32] Pérez MJ, Falqué E, Domínguez H. Antimicrobial action of compounds from marine seaweed. Mar Drugs 2016;14:52.

[33] Vanegas CH, Hernon A, Bartlett J. Enzymatic and organic acid pretreatment of seaweed: effect on reducing sugars production and on biogas inhibition. Int J Ambient Energy 2015;36:2-7.

[34] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: A review. Bioresour Technol 2008;99:4044-64.

[35] Xia A, Cheng J, Murphy JD. Innovation in biological production and upgrading of methane and hydrogen for use as gaseous transport biofuel. Biotechnol Adv 2015.

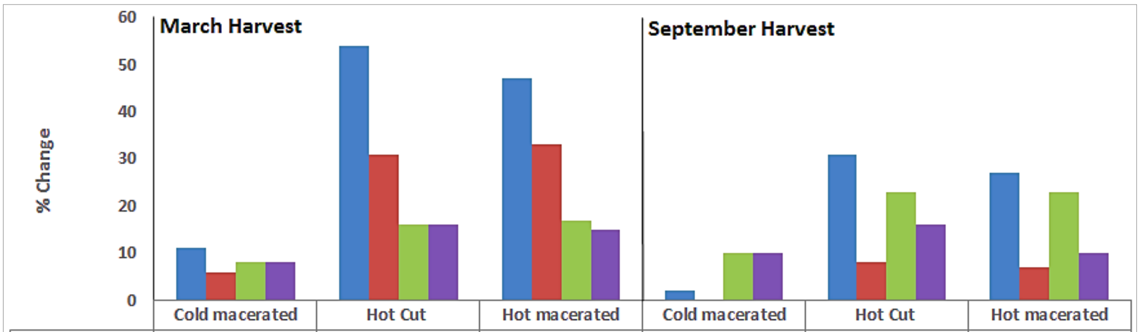
[36] Fang C, Boe K, Angelidaki I. Anaerobic co-digestion of desugared molasses with cow manure; focusing on sodium and potassium inhibition. Bioresour Technol 2011;102:1005-11.

[37] Monlau F, Sambusiti C, Barakat A, Quéméneur M, Trably E, Steyer JP, et al. Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review. Biotechnol Adv 2014;32:934-51.

[38] Wall DM, O'Kiely P, Murphy JD. The potential for biomethane from grass and slurry to satisfy renewable energy targets. Bioresour Technol 2013;149:425-31.

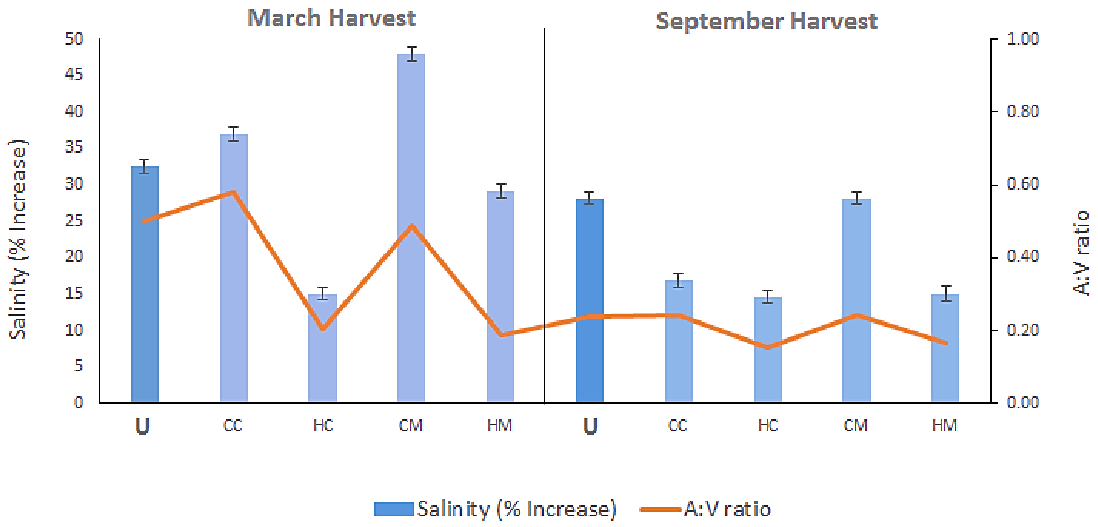
[39] Browne JD, Allen E, Murphy JD. Assessing the variability in biomethane production from the organic fraction of municipal solid waste in batch and continuous operation. Appl Energy 2014;128:307-14.

List of Figures and Tables



	Cold macerated	Hot Cut	Hot macerated	Cold macerated	Hot Cut	Hot macerated
Ash removal	11	54	47	2	31	27
OC improved	6	31	33	0	8	7
BI enhanced	8	16	17	10	23	23
BMP Increased	8	16	15	10	16	10

Fig.1. Impact of various pre-treatment on the process parameters of *L. digitata*



U untreated; C cold cut; CM cold macerated; HC hot cut; HM hot macerated

Fig. 2. Effect of pre-treatment on salts accumulation in the batch process.

Table 1 Change in the chemistry of *L. digitata* after different pre-treatments

Pre-treatment	Compositional Analysis					Elemental Analysis				
	TS (%)	VS (%)	OMC (%)	Ash (%)	A:V	C %	H %	N %	O %	C:N
March										
Untreated	9.74 (0.02)	6.49 (0.14)	66.67	33.33 (1.37)	0.51	30.41 (0.90)	3.97 (0.11)	3.70 (0.06)	28.58	8.22
Cold cut	9.04 (0.20)	5.72 (0.20)	63.26	36.73 (0.50)	0.58	28.09 (0.38)	3.46 (0.07)	3.55 (0.34)	28.16	7.91
Hot cut	6.47 (0.06)	5.37 (0.09)	83.09	16.91 (0.06)	0.20	39.29 (0.12)	4.83 (0.05)	2.84 (0.05)	36.12	13.83
Cold macerated	9.76 (0.12)	6.56 (0.07)	67.19	32.81 (0.05)	0.49	30.41 (0.90)	3.97 (0.11)	3.70 (0.57)	29.10	8.22
Hot macerated	8.32 (0.10)	7.02 (0.02)	84.38	15.62 (0.7)	0.19	39.53 (0.01)	4.88 (0.12)	2.91 (0.29)	37.06	13.58
September										
Untreated	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	39.37	39.38
Cold cut	19.44 (0.34)	15.60 (0.35)	80.27	19.43 (0.43)	0.24	36.74 (0.17)	5.03 (0.11)	1.18 (0.08)	37.32	31.14
Hot cut	15.51 (0.36)	13.42 (0.32)	86.56	13.44 (0.30)	0.16	38.98 (0.17)	5.21 (0.11)	0.97 (0.25)	41.39	40.19
Cold macerated	19.46 (0.26)	15.67 (0.25)	80.51	19.49 (0.44)	0.24	36.62 (0.17)	5.30 (0.05)	0.93 (0.03)	37.66	39.38
Hot macerated	16.82 (0.10)	14.42 (0.10)	85.75	14.25 (0.14)	0.17	38.20 (0.07)	5.39 (0.02)	0.93 (0.10)	41.23	41.08

TS is total solids, VS is volatile solids, OMC is organic matter content obtained dividing VS/TS, A:V is ash to volatile solid ratio, while C, H, N, O and C:N are carbon, hydrogen, nitrogen, oxygen and carbon to nitrogen ratio, respectively. Standard deviation is in parentheses

Table 2 Effect of pre-treatments on the gas yield and specific yield production

Pre-treatment	**BMP yield** ($L\ CH_4\ kg\ VS^{-1}$)	**TMP** ($L\ CH_4\ kg\ VS^{-1}$)	**BI** (BMP/TMP)	**Specific yield** ($m^3\ CH_4\ t\ wwt^{-1}$)
March				
Untreated	245 (10.86)	469	0.52	16
Cold cut	258 (12.42)	469	0.55	15
Hot cut	283 (6.24)	468	0.60	15
Cold macerated	265 (3.56)	469	0.57	17
Hot macerated	282 (2.33)	462	0.61	20
September				
Untreated	280 (28.76)	450	0.62	44
Cold cut	303 (22.55)	450	0.67	47
Hot cut	326 (26.25)	424	0.76	44
Cold macerated	307 (18.98)	450	0.68	48
Hot macerated	308 (5.63)	403	0.76	44

BMP, TMP, BI and wwt are biomethane potential, theoretical methane potential, biodegradability and wet weight, respectively. Standard deviation is in parentheses.
Sample calculation for specific methane yield:
Specific yield (March, un-treated) = 0.245 $m^3\ CH_4\ t\ VS^{-1}$ × 64.9 (kg VS per t wwt, (Table 1)) = 16 $m^3\ CH_4\ t\ wwt^{-1}$

Table 3 The process dynamics of *L. digitata* based on different pre-treatments

Pre-treatment	***K* (days^{-1})**	**R^2**	**Y_{max}**	**T_{50} (days)**
March				
Untreated	0.10	0.98	245	6.81
Cold cut	0.08	0.97	258	8.89
Hot cut	0.08	0.96	283	8.43
Cold macerated	0.10	0.97	265	6.81
Hot macerated	0.20	0.99	282	3.46
September				
Untreated	0.13	0.95	282	5.24
Cold cut	0.15	0.99	338	4.68
Hot cut	0.08	0.95	326	8.20
Cold macerated	0.13	0.96	307	5.24
Hot macerated	0.09	0.93	308	7.49
Cellulose	0.17	0.99	356	4.09

k is the decay constant, R^2 is a measure that how the kinetic model fits the biomethane potential curve (%), Y_{max} is the maximum methane potential and T_{50} is the half-life methane production (days).

- Maceration after hot washing of *L. digitata* significantly affected biogas yield.
- Impact of the pre-treatment was more significant on March harvest than September.
- The pre-treatment removed 54% of ash whilst increasing VS content by 31%.
- Bio-digestibility was enhanced by 16% with 54% less salt accumulation in digester.
- Specific methane yield of *L. digitata* per weight wet was increased by 25%.